

# Figure 1A

2H7scFv-Ig cDNA and predicted amino acid sequence:

HindIII      NcoI      2H7 V<sub>L</sub> Leader Peptide →  
~~~~~ ~~~~~~  
1    **AAGCTT**GCCG CC    ATGGATT TCAAGTCAG ATTTCAGCT TCCTGCTAAT CAGTGCTTCA  
  
2H7 V<sub>L</sub> →  
61    V I I A R G Q I V L S Q S P A I L S A S  
GTCATAATTG CCAGAGGACA ATTGTTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT  
  
121    P G E K V T M T C R A S S S V S Y M H W  
CCAGGGGAGA AGGTACAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG  
  
BamHI  
~~~~~  
181    Y Q Q K P G S S P K P W I Y A P S N L A  
TACCAGCAGA AGCCAGGATC CTCCCCAAA CCCTGGATT ATGCCCATC CAACCTGGCT  
  
241    S G V P A R F S G S G S G T S Y S L T I  
TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAAATC  
  
301    S R V E A E D A A T Y Y C Q Q W S F N P  
AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTJTAACCCA  
  
(Gly<sub>4</sub>Ser)<sub>3</sub> Linker  
P T F G A G T K L E L K G G G S G G G  
CCCACGTTCG GTGCTGGAC CAAGCTGGAG CTGAAAGGTG CGGGTGGCTC GGGCGGTGGT  
  
2H7 V<sub>H</sub> →  
421    G S G G G G S S Q A Y L Q Q S G A E L V  
GGATCTGGAG GAGGTGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGC TGAGCTGGTC  
  
481    R P G A S V K M S C K A S G Y T F T S Y  
AGGCCTGGGG CCTCACTGAA CATGTCCTGC AAGGCTCTG GCTACACATT TACCACTTAC  
  
541    N M H W V K Q T P R Q G L E W I G A I Y  
AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTAT  
  
601    P G N G D T S Y N Q K F K G K A T L T V  
CCAGGAAATG GTCATCTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA  
  
661    D K S S S T A Y M Q L S S L T S E D S A  
GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGG  
  
721    V Y F C A R V V Y Y S N S Y W Y F D V W  
GTCTATTCT GTGCAAGAGT GGTGTACTAT AGTAACCTTT ACTGGTACTT CGATGTCTGG

**Figure 1 B**

BcI I  
~~~~~human IgG1 Fc domain →

781 G T G T T V T V S D Q E P K S C D K T H  
GGCACAGGGA CCACGGTCAC CGTCTCT**GAT** CAGGAGCCCA AATCTGTGA CAAAACTCAC

841 T C P P C P A P E L L G G P S V F L F P  
ACATGCCAC CGTCCCCAGC ACCTGAACTC CTGGGGGAC CGTCAGTCTT CCTCTTCCCC

901 P K P K D T L M I S R T P E V T C V V V  
CCAAAACCCA AGGACACCCT CATGATCTCC CGGACCCCTG AGGTCACATG CGTGGTGGTG

961 D V S H E D P E V K F N W Y V D G V E V  
GACGTGAGCC ACGAAGACCC TGAGGTCAAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG

1021 H N A K T K P R E E Q Y N S T Y R V V S  
CATAAATGCCA AGACAAAGCC GCAGGGAGGAG CAGTACAACA GCACGTACCG TGTGGTCAGC

1081 V L T V L H Q D W L N G K E Y K C K V S  
GTCCTCACCG TCCTGCACCA GGACTGGCTG AATGGCAAGG AGTACAAGTG CAAGGGTCTCC

1141 N K A L P A P I E K T I S K A K G Q P R  
AACAAAGCCC TCCCAGCCC CATCGAGAAA ACAATCTCCA AAGCCAAAGG GCAGCCCCGA

1201 E P Q V Y T L P P S R D E L T K N Q V S  
GAACCACAGG TGTACACCCT GCCCCCCATCC CGGGATGAGC TGACCAAGAA CCAGGTCAGC

1261 L T C L V K G F Y P S D I A V E W E S N  
CTGACCTGCC TGGTCAAAGG CTTCTATCCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT

1321 G Q P E N N Y K T T P P V L D S D G S F  
GGGCAGCCGG AGAACAACTA CAAGACCACCG CCTCCCGTGC TGGACTCCGA CGGCTCCTTC

1381 F L Y S K L T V D K S R W Q Q G N V F S  
TTCCTCTACA GCAAGCTCAC CGTGGACAAG AGCAGGTGGC AGCAGGGGAA CGTCTTCTCA

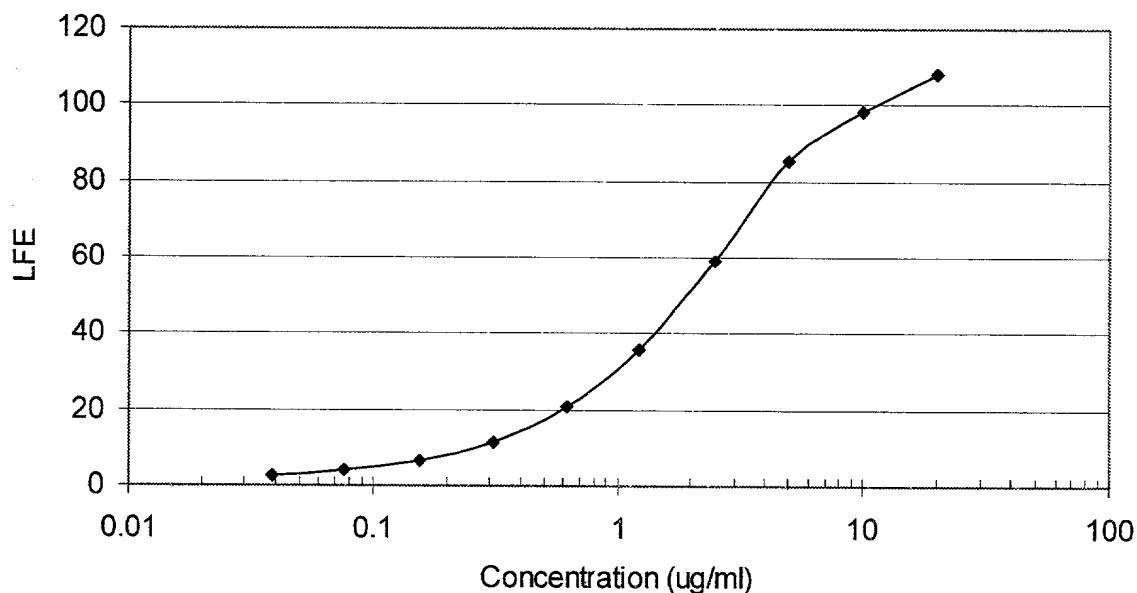
1441 C S V M H E A L H N H Y T Q K S L S L S  
TGCTCCGTGA TGCATGAGGC TCTGCACAAC CACTACACGC AGAAGAGCCT CTCCCTGTCT

XbaI  
~~~~~

1501 P G K \* S R  
CCGGGTAAAT **GATCTAGA**

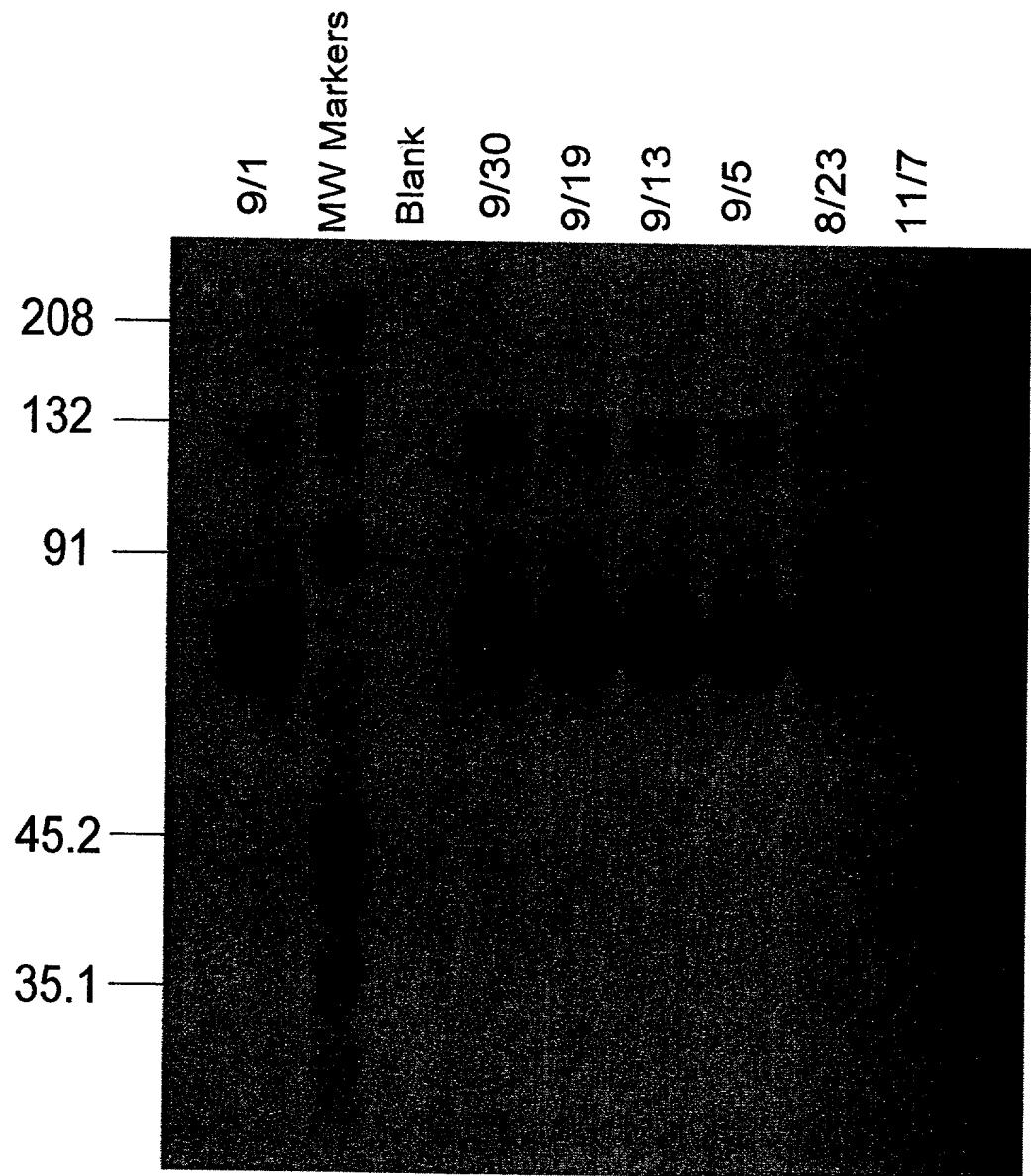
## Figure 2.

2H7scFvIg Standard Curve



Clone	LFE @ 1:50	Estimated Concentration (μg/ml)
D2	26.1	56
IIIC6	25.7	55
IVA3	28.6	61
Spent bulk	29.6	64

**Figure 3.**



## Figure 4A.

Complement Mediated B Cell Killing After Binding of CD20-targeted 2H7 Derivatives:

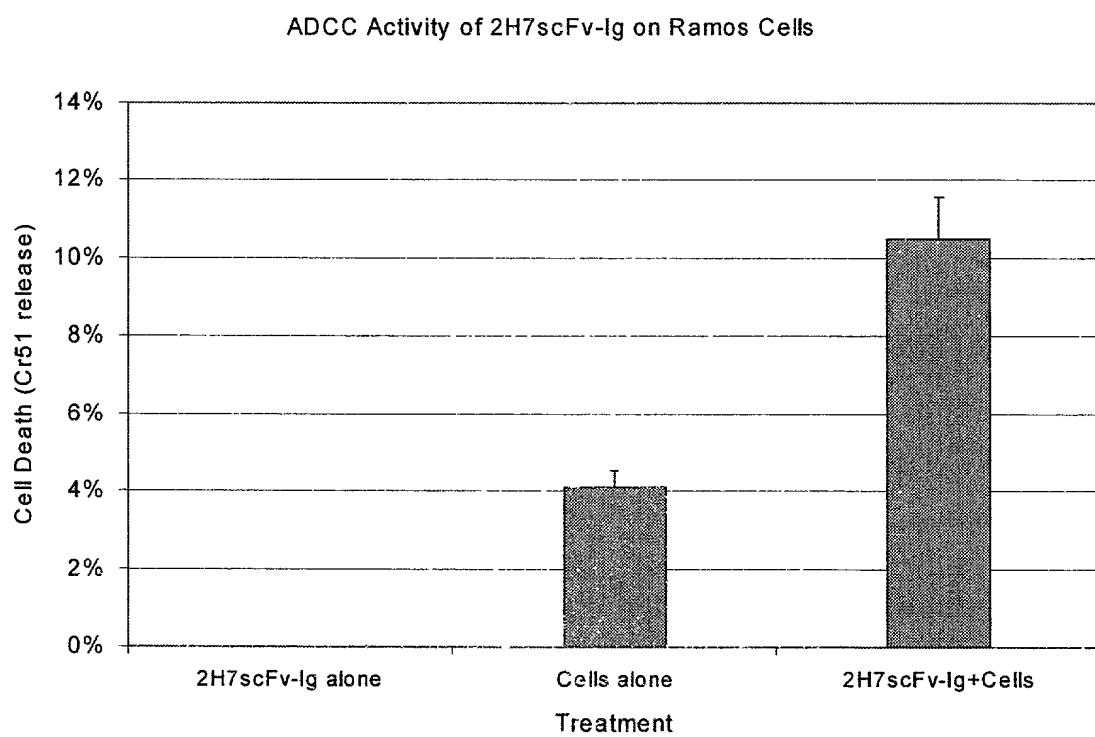
2H7scFv-Ig Concentration	RAMOS	BJAB
20 µg/ml + complement	0.16	0.07
5 µg/ml + complement	0.2	N.D.
1.25 µg/ml + complement	0.32	0.1
Complement alone	0.98	0.94

\*Viability was determined by trypan blue exclusion and is tabulated as the fraction of viable cells out of the total number of cells counted.

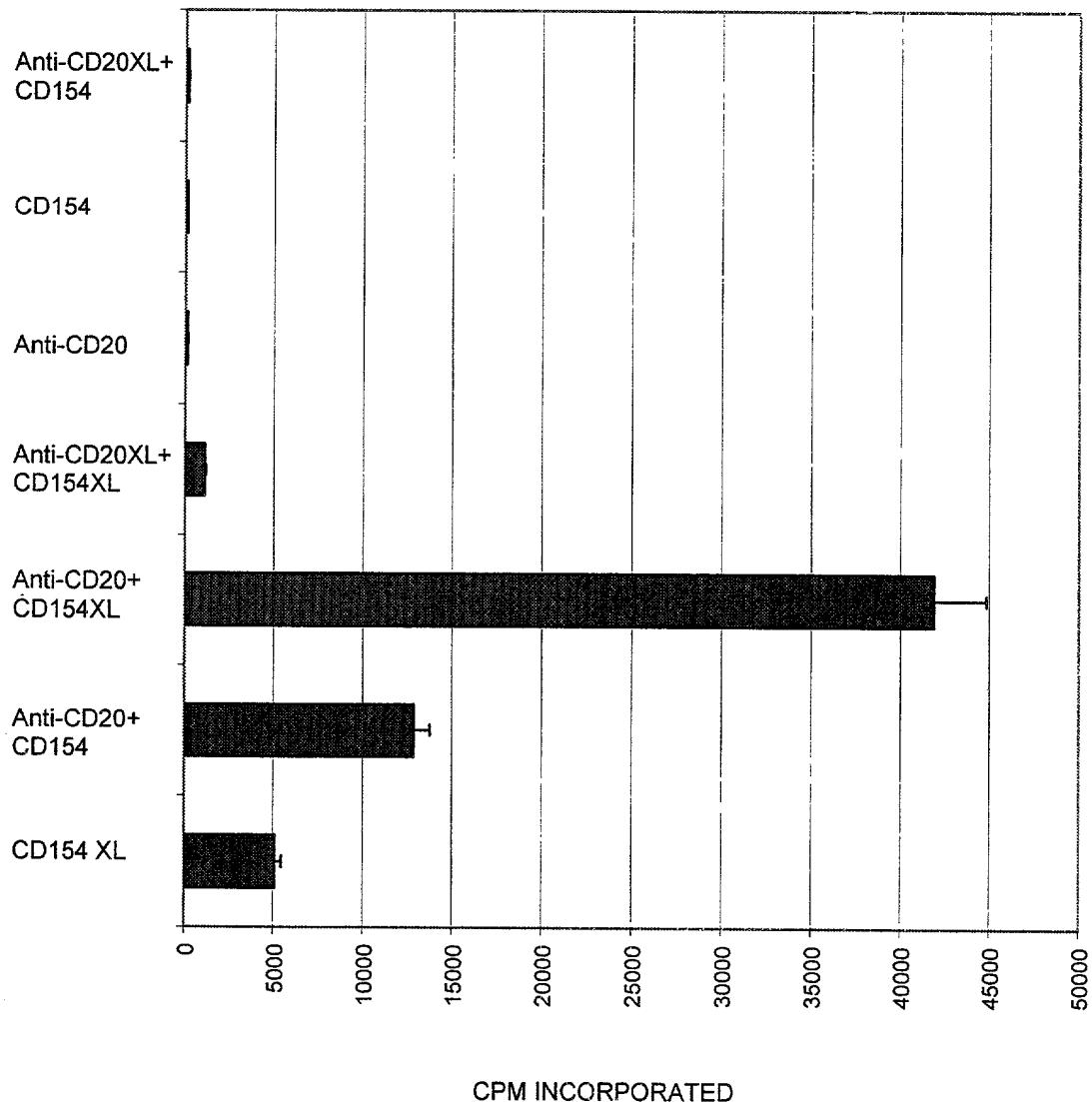
\*\*N.D. (not determined).

## Figure 4B.

Antibody-dependent cellular cytotoxicity (ADCC) mediated by 2H7scFv-Ig:

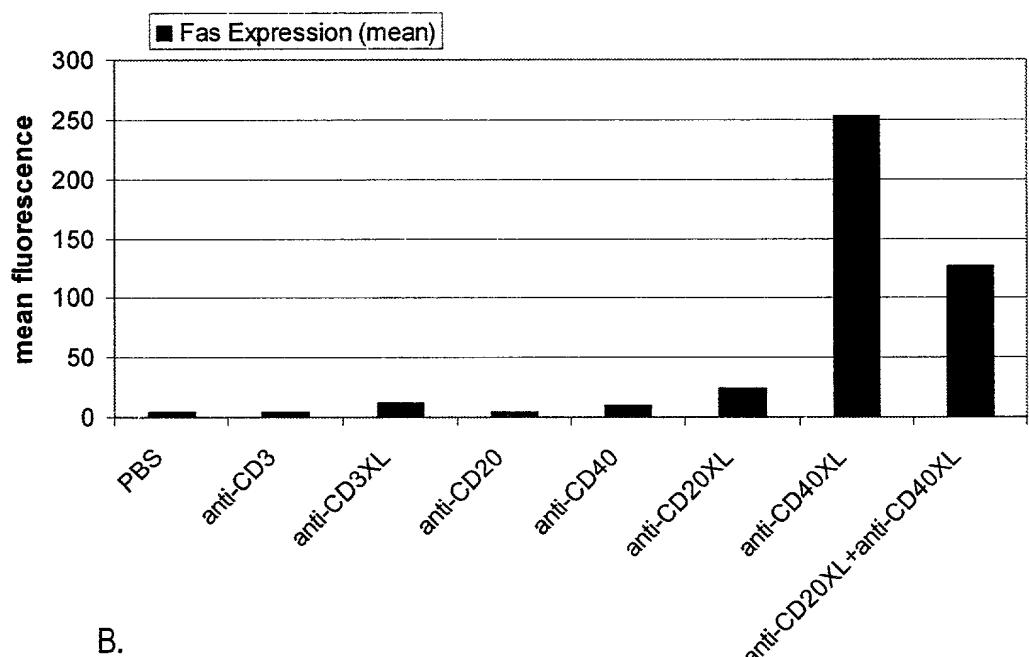


**Figure 5.**

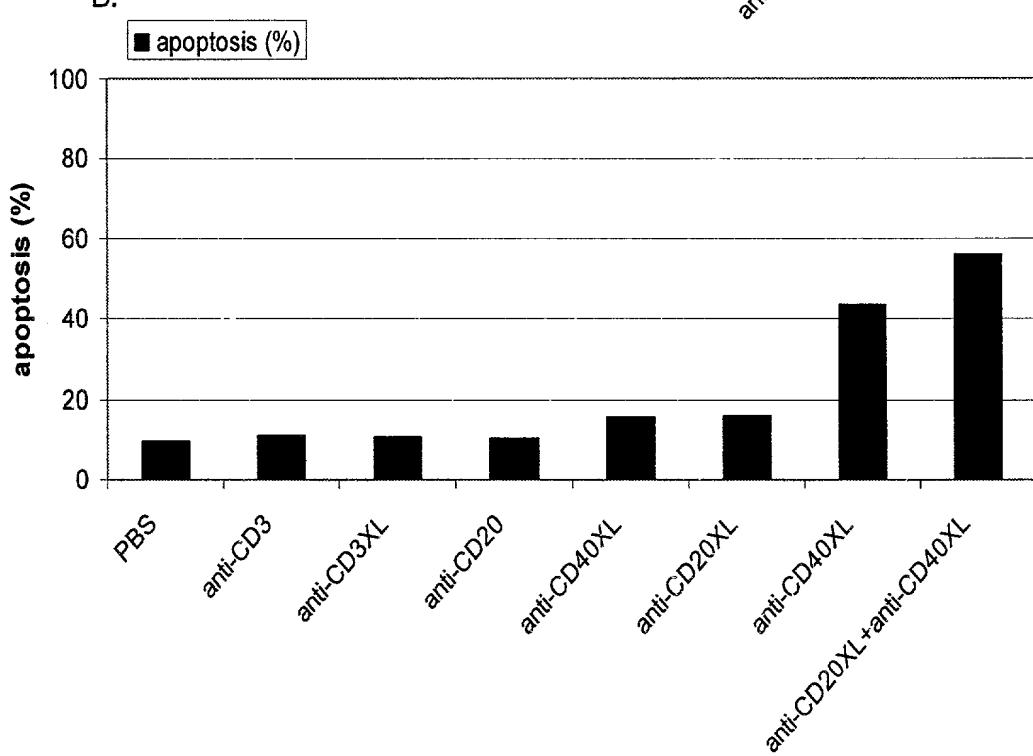


**Figure 6A and B.**

**A.**



**B.**



## Figure 7A.

### 2H7-CD154 L2 cDNA and predicted amino acid sequence:

HindIII      NcoI      2H7 V<sub>L</sub> Leader Peptide →  
~~~~~ ~~~~~~  
1    **AAGCTT**GCCG CC     ATGGATT TCAAGTCAG ATTTCAGCT TCCTGCTAA CAGTGCTTCA

2H7 V<sub>L</sub> →  
V I I A R G Q I V L S Q S P A I L S A S  
61    GTCATAATTG CCAGAGGACA AATTGTTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT

P G E K V T M T C R A S S S V S Y M H W  
121    CCAGGGGAGA AGGTACAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG

BamHI  
~~~~~  
181    Y Q Q K P G S S P K P W I Y A P S N L A  
TACCAAGCAGA AGCCAGGATC CTCCCCAAA CCTGGATTG ATGCCCATC CAACCTGGCT

S G V P A R F S G S G S G T S Y S L T I  
241    TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAAATC

S R V E A E D A A T Y Y C Q Q W S F N P  
301    AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTAACCCA

(Gly<sub>4</sub>Ser)<sub>3</sub> Linker →  
P T F G A G T K L E L K G G G G S G G G  
361    CCCACGTTCG GTGCTGGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT

2H7 V<sub>H</sub> →  
G S G G G G S S Q A Y L Q Q S G A E L V  
421    GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGGC TGAGCTGGTG

R P G A S V K M S C K A S G Y T F T S Y  
481    AGGCCTGGGG CCTCAGTGAA GATGTCCTGC AAGGCTTCTG GCTACACATT TACCAGTTAC

N M H W V K Q T P R Q G L E W I G A I Y  
541    AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTTAT

P G N G D T S Y N Q K F K G K A T L T V  
601    CCAGGAAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA

D K S S S T A Y M Q L S S I T S E D S A  
661    GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG

V Y F C A R V V Y Y S N S Y W Y F D V W  
721    GTCTATTCT GTGCAAGAGT GGTGTACTAT ACTAACTCTT ACTGGTACTT CGATGCTGG

**Figure 7B**

human CD154/amino acid 48→

Bcl/Bam hybrid site

781 G T G T T V T V S D P R R L D K I E D E  
GGCACAGGGA CCACGGTCAC CGTCTCT**TGAT** CCAAGAAGGT TGGACAAGAT AGAAGATGAA

841 R N L H E D F V F M K T I Q R C N T G E  
AGGAATCTC ATGAAGATTG TGTATTCATG AAAACGATAAC AGAGATGCAA CACAGGAGAA

901 R S L S L L N C E E I K S Q F E G F V K  
AGATCCTTAT CCTTACTGAA CTGTGAGGAG ATTAAAAGCC AGTTTGAGG CTTTGTGAAG

BclI

961 D I M L N K E E T K K E N S F E M Q K G  
GATATAATGT TAAACAAAGA GGAGACGAAG AAAGAAAACA GCTTGAAAT GCAAAAGGT

BclI

~~~~~

1021 D Q N P Q I A A H V I S E A S S K T T S  
GATCAGAACATC CTCAAATTGC GGCACATGTC ATAAGTGAGG CCAGCAGTAA AACAAACATCT

1081 V L Q W A E K G Y Y T M S N N L V T L E  
GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTTGTT AACCTGGAA

1141 N G K Q L T V K R Q G L Y Y I Y A Q V T  
AATGGGAAAC AGCTGACCGT TAAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC

HindIII

~~~~~

1201 F C S N R E A S S Q A P F I A S L C L K  
TTCTGTTCCA ATCGGGAAAGC TTCGAGTCAT GCTCCATTAA TAGCCAGCCT CTGCCTAAAG

1261 S P G R F E R I L L R A A N T H S S A K  
TCCCCCGGTA GATTGAGAG AATCTTACTC AGAGCTGCAA ATACCCACAG TTCCGCCAAA

1321 P C G Q Q S I H L G G V F E L Q P G A S  
CCTTGCAGGC AACAAATCCAT TCACCTGGGA GGAGTATTG AATTGCAACC AGGTGCTTCG

NcoI

~~~~~

1381 V F V N V T D P S Q V S H G T G F T S F  
GTGTTGTCA ATGTGACTGA TCCAAGCCAA GTGAGCCATG GCACGGCTT CACGTCCCTT

XbaI

~~~~~

XbaI

~~~~~

1441 G L L K L E \* \* S R  
GGCTTACTCA AACTCGAGTG ATAAT**CTAGA**

## Figure 7 C

**2H7scFv-CD154 S4 cDNA and predicted amino acid sequence:**

HindIII      NcoI  
~~~~~ ~~~~~~**2H7 V<sub>L</sub> Leader Peptide→**  
1    AAGCTTGCCG CC    ATGGATT TCAAGTCAG ATTTCAGCT TCCTGCTAAT CAGTGCTTCA  
  
**2H7 V<sub>L</sub> →**  
61    V I I A R G Q I V L S Q S P A I L S A S  
      GTCATAATTG CCAGAGGACA ATTGTTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT  
  
121    P G E K V T M T C R A S S S V S Y M H W  
      CCAGGGGAGA AGGTACAAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG  
  
BamHI  
~~~~~  
181    Y Q Q K P G S S P K P W I Y A P S N L A  
      TACCAGCAGA AGCCAGGATC CTCCCCAAA CCCTGGATTT ATGCCCATC CAACCTGGCT  
  
241    S G V P A R F S G S G S G T S Y S L T I  
      TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAAATC  
  
301    S R V E A E D A A T Y Y C Q Q W S F N P  
      AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTAACCCA  
  
**(Gly<sub>4</sub>Ser)<sub>3</sub> Linker →**  
361    P T F G A G T K L E L K G G G G S G G G  
      CCCACGTTCG GTGCTGGAC CAAGCTGGAG CTGAAAGGTG CGGGTGGCTC GGGCGGTGGT  
  
**2H7 V<sub>H</sub> →**  
421    G S G G G G S S Q A Y L Q Q S G A E L V  
      GGATCTGGAG CAGGTGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGC TGAGCTGGTG  
  
481    R P G A S V K M S C K A S G Y T F T S Y  
      AGGCCTGGGG CCTCAGTGAA GATGTCCTGC AAGGCTCTG GCTACACATT TACCAAGTTAC  
  
541    N M H W V K Q T P R Q G L E W I G A I Y  
      AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTAT  
  
601    P G N G D T S Y N Q K F K G K A T L T V  
      CCAGGAAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA  
  
661    D K S S S T A Y M Q L S S L T S E D S A  
      GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG  
  
721    V Y F C A R V V Y Y S N S Y W Y F D V W  
      GTCTATTTCT GTGCAAGAGT GGTGTACTAT AGTAACCTT ACTGGTACTT CGATGTCTGG

**Figure 7D.**

human CD154/amino acid 108 →

BclI/Bam hybrid site

BclI

781 G T G T T V T V S D P E N S F E M Q K G  
GGCACAGGG A CCACGGTCAC CGTCTC **TGAT** CCAGAAAACA GCTTTGAAAT GCAAAAAGGT

BclI  
~~~~~

841 D Q N P Q I A A H V I S E A S S K T T S  
GATCAGAAC TCTCAAATTGC GGCACATGTC ATAAGTGAGG CCAGCAGTAA AACAACATCT

901 V L Q W A E K G Y Y T M S N N L V T L E  
GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTTGGT AACCCCTGGAA

961 N G K Q L T V K R Q G L Y Y I Y A Q V T  
AATGGGAAAC AGCTGACCGT TAAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC

HindIII

1021 F C S N R E A S S Q A P F I A S L C L K  
TTCTGTTCCA ATCGGGAAAGC TTGAGTCAA GCTCCATTAA TAGCCAGCCT CTGCCTAAAG

1081 S P G R F E R I L L R A A N T H S S A K  
TCCCCCGGTA GATTGAGAG AATCTTACTC AGAGCTGCAA ATACCCACAG TTCCGCCAAA

1141 P C G Q Q S I H L G G V F E L Q P G A S  
CCTTGCGGGC AACAAATCCAT TCACTTGGGA GGAGTATTTG AATTGCAACC AGGTGCTTCG

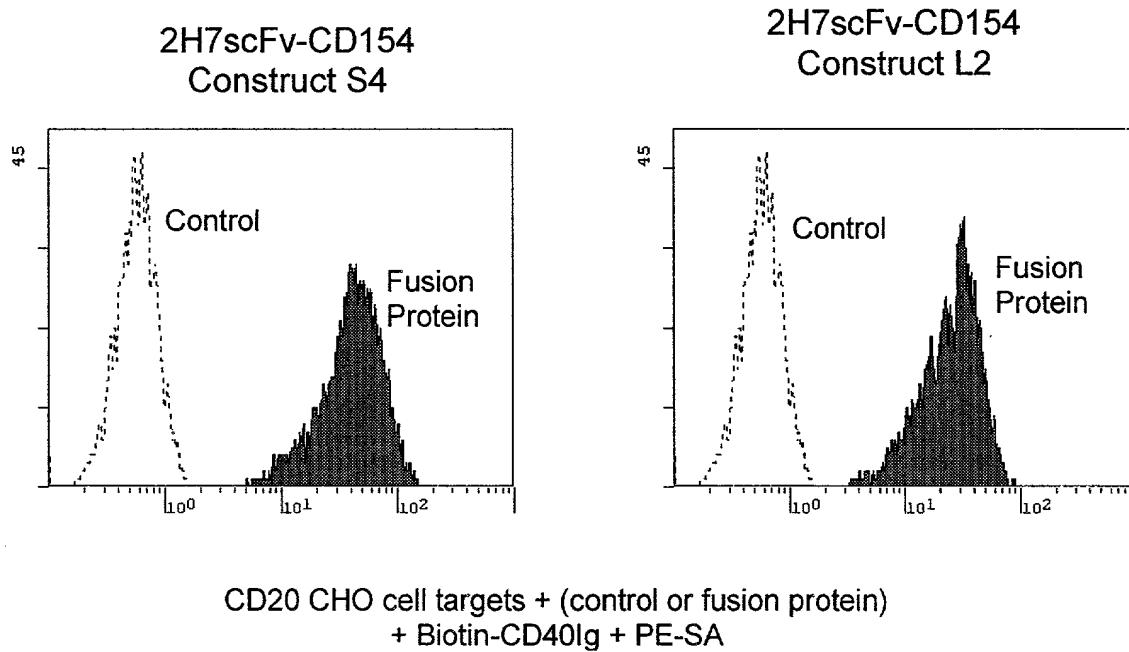
NcoI

1201 V F V N V T D P S Q V S H G T G F T S F  
GTGTTGTCA ATGTGACTGA TCCAAGCCAA GTGAGCCATG GCACTGGCTT CACGTCCCTT

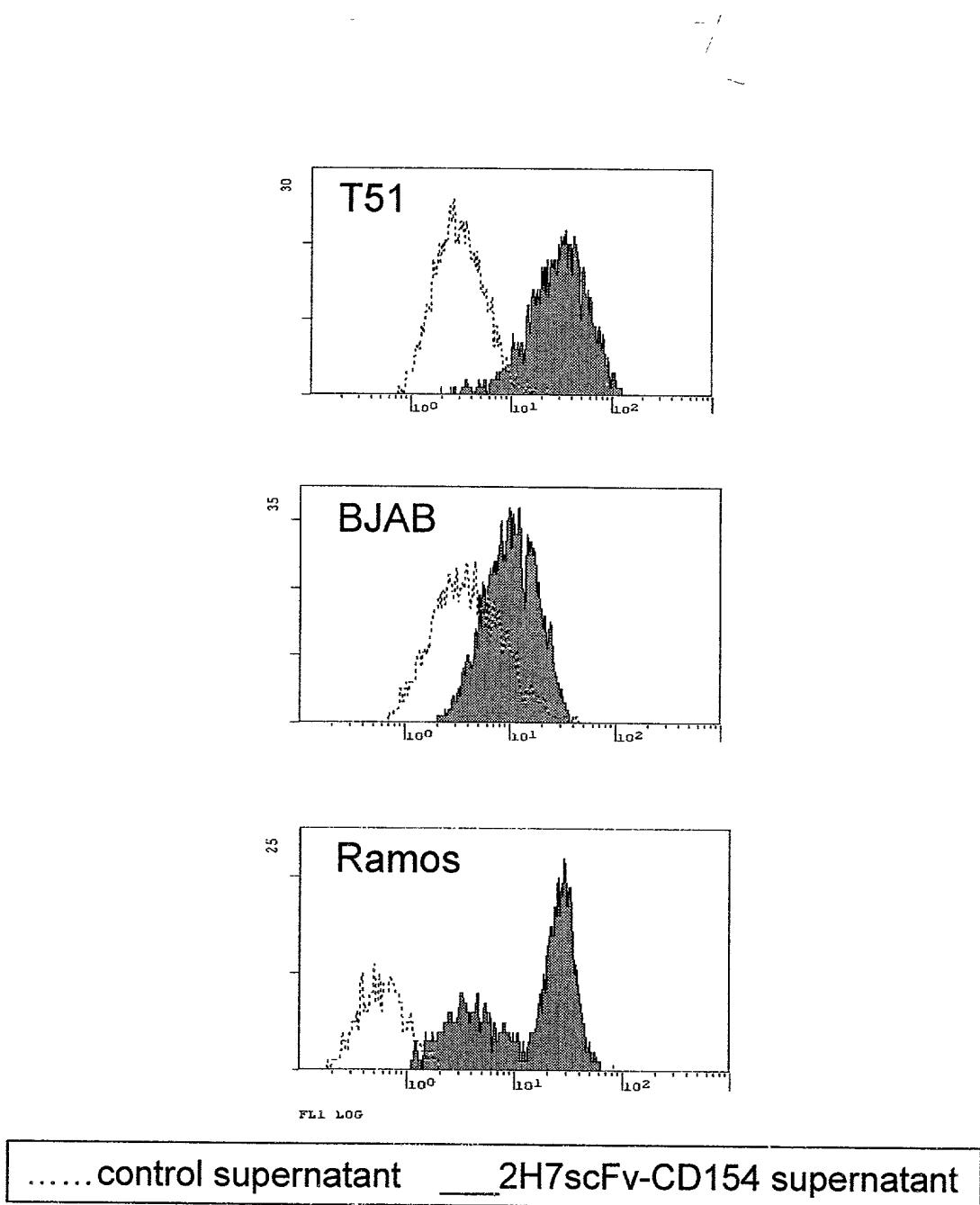
XbaI

1261 XbaI  
~~~~~  
G L L K L E \* \* S R  
GGCTTACTCA AACTCGAGTG ATAATCTAGA

**Figure 8.**



**Figure 9.**



**Figure 10.**

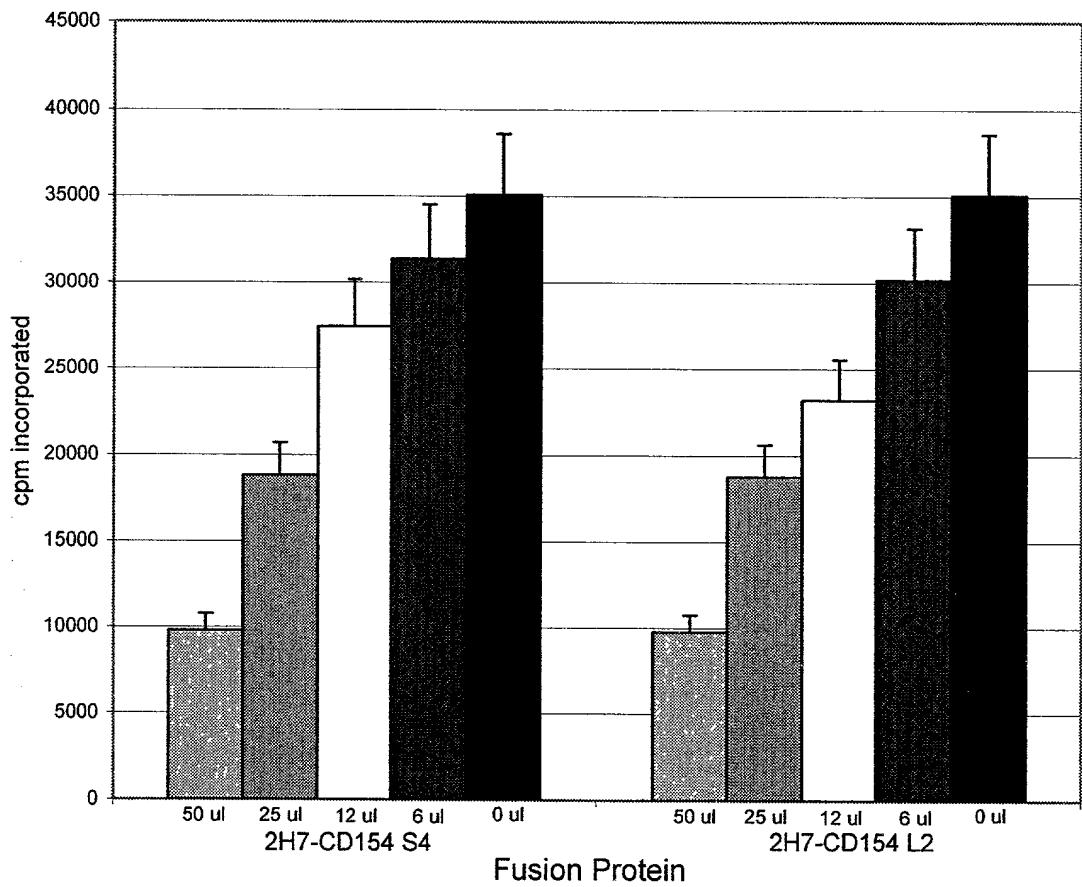
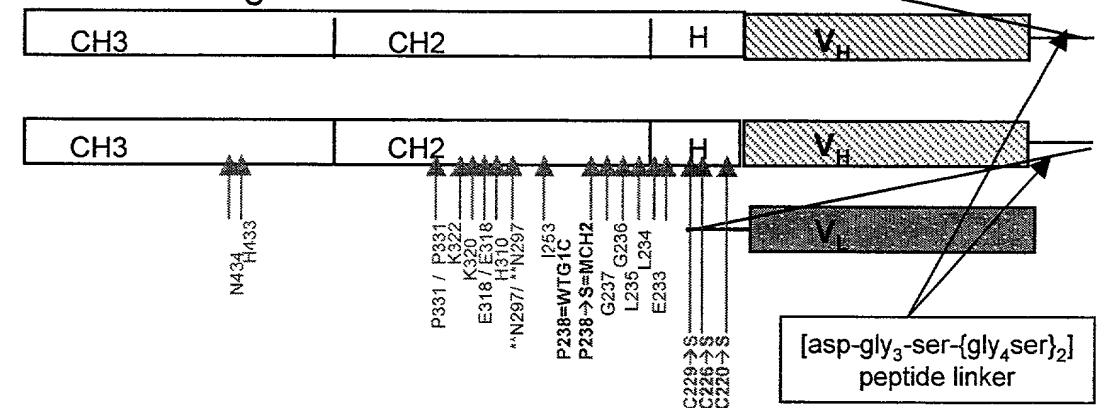


FIGURE 11

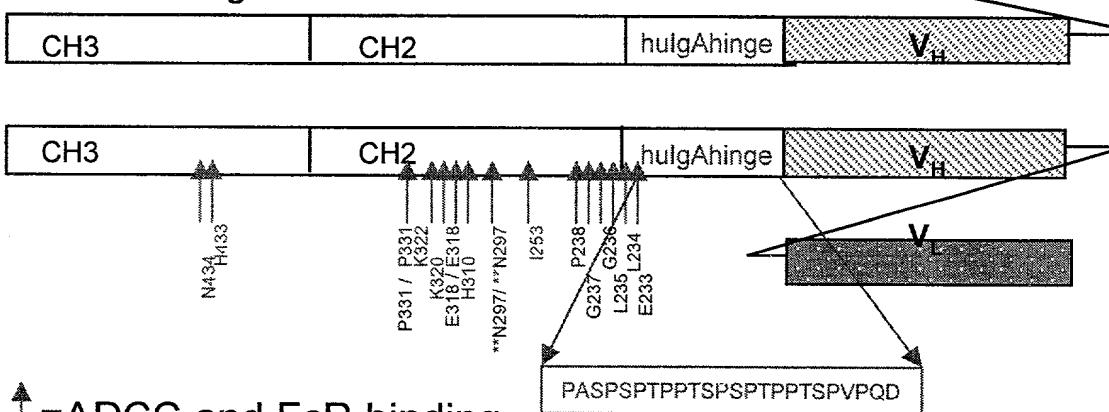
CytoxB-MHWTG1C  
OR CytoxB-MHMG1C:

Human IgG1 CH2-CH3



CytoxB-IgAHWTHG1C:

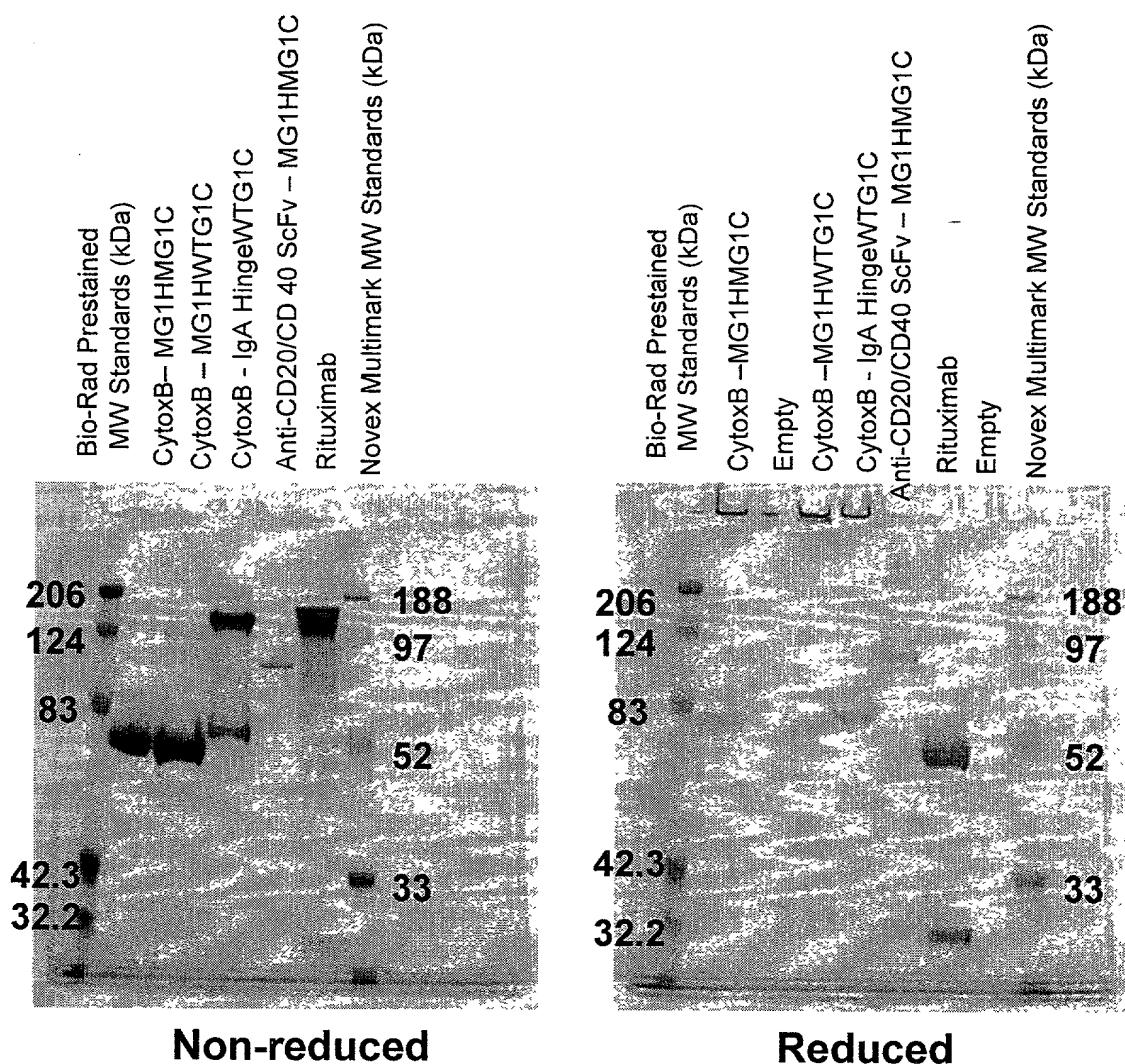
Human IgG1 CH2-CH3



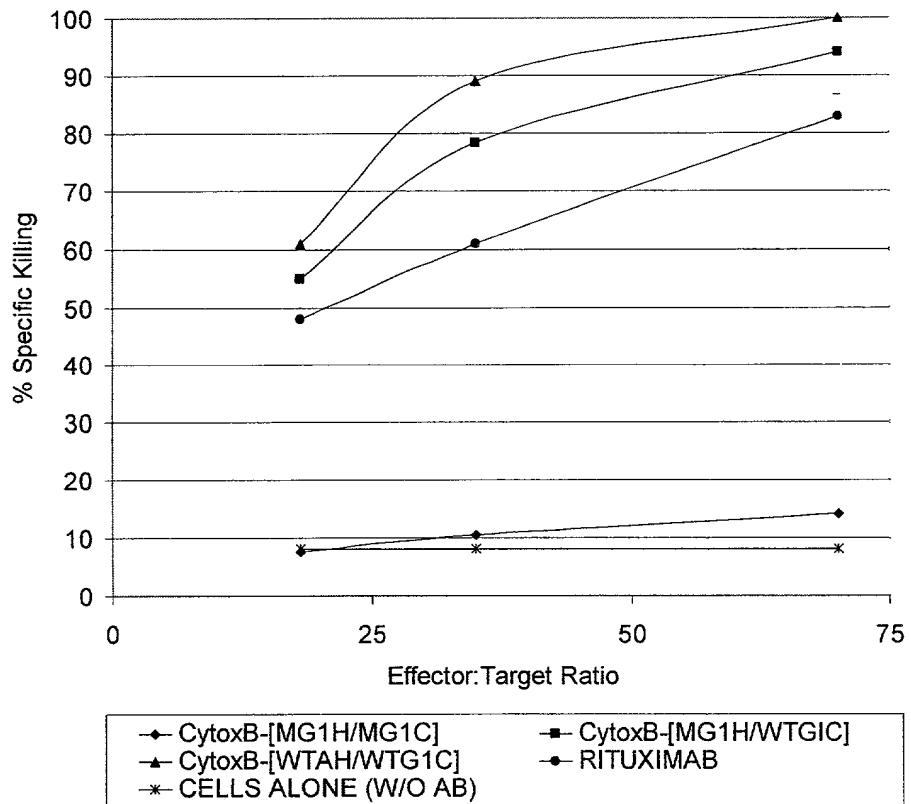
↑ =ADCC and FcR binding

↑ =Complement Fixation

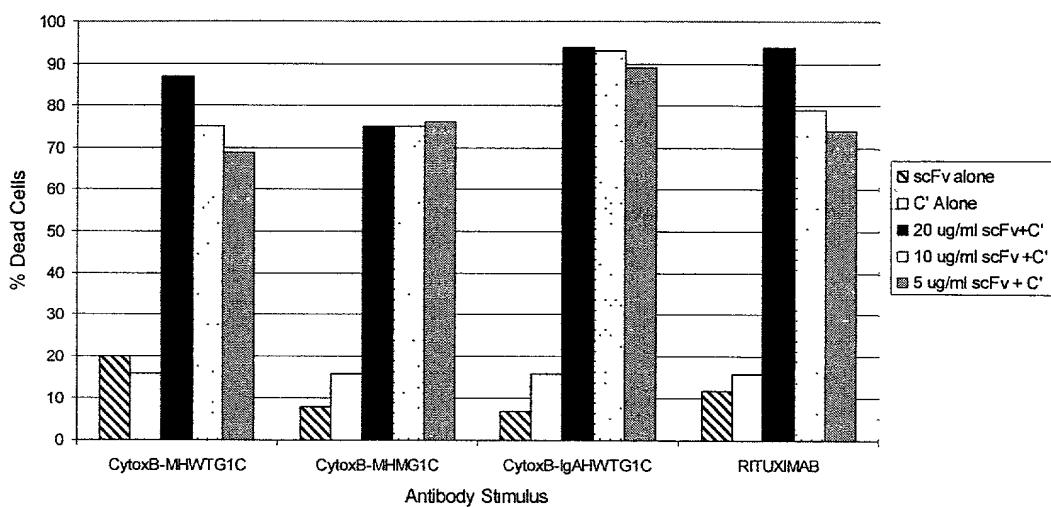
**FIGURE 12**



**FIGURE 13**



**FIGURE 14**

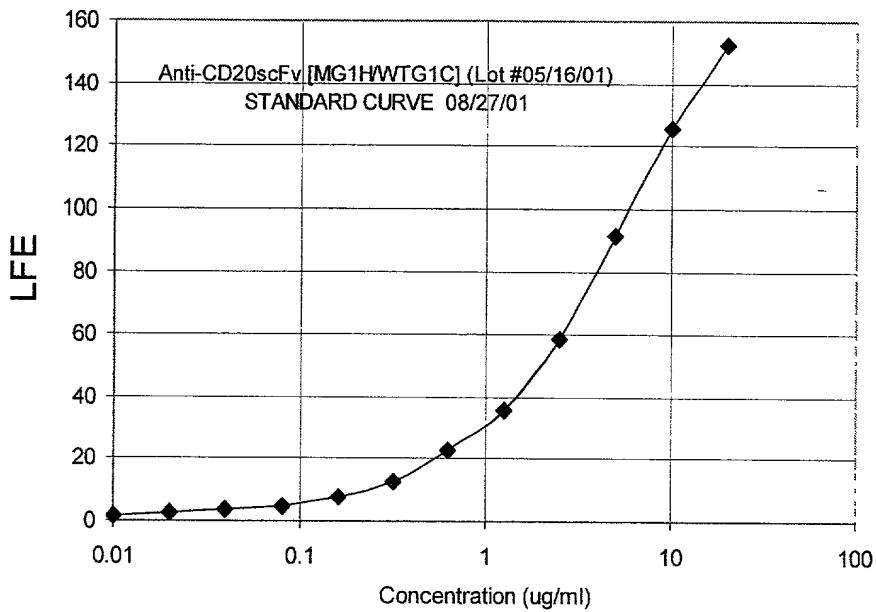


**TITLE: BINDING DOMAIN-IMMUNOGLOBULIN FUSION PROTEINS**

Inventors: Jeffrey A. Ledbetter et al. Docket No. 390069.401

EXPRESS MAIL NO. EL 755733415US

FIGURE 15



Day	Monkey J99231		Monkey K99334	
	LFE(1:40)	Concentration ( $\mu$ g/mL)	LFE(1:40)	Concentration ( $\mu$ g/mL)
Injection → -7	2.41	<0.6 $\mu$ g/mL	1.51	<0.4 $\mu$ g/mL
0	2.22	<0.6 $\mu$ g/mL	1.63	<0.4 $\mu$ g/mL
Injection → 1	73.8	220 $\mu$ g/mL	44.4	100 $\mu$ g/mL
3	20.0	28 $\mu$ g/mL	40.2	80 $\mu$ g/mL
7	15.6	24 $\mu$ g/mL	15.7	24 $\mu$ g/mL
8	39.1	80 $\mu$ g/mL	42.6	92 $\mu$ g/mL
10	11.5	18 $\mu$ g/mL	2.74	1.2 $\mu$ g/mL
14	2.05	0.6mg/mL	1.96	0.6 $\mu$ g/mL

**Figure 16**

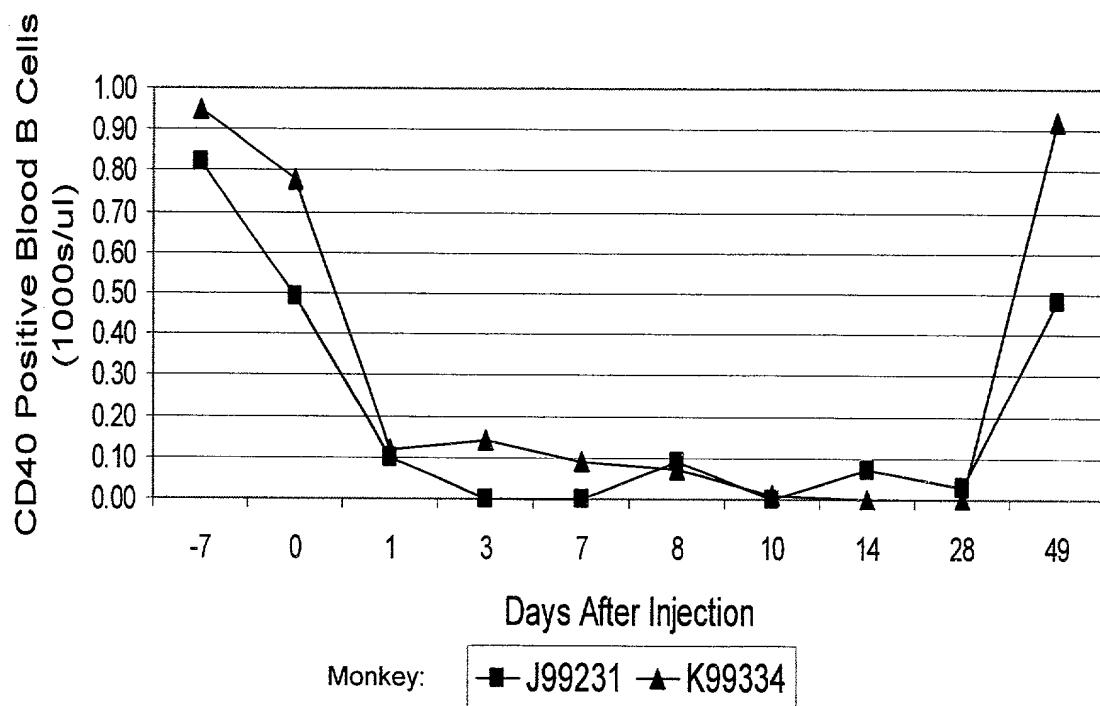
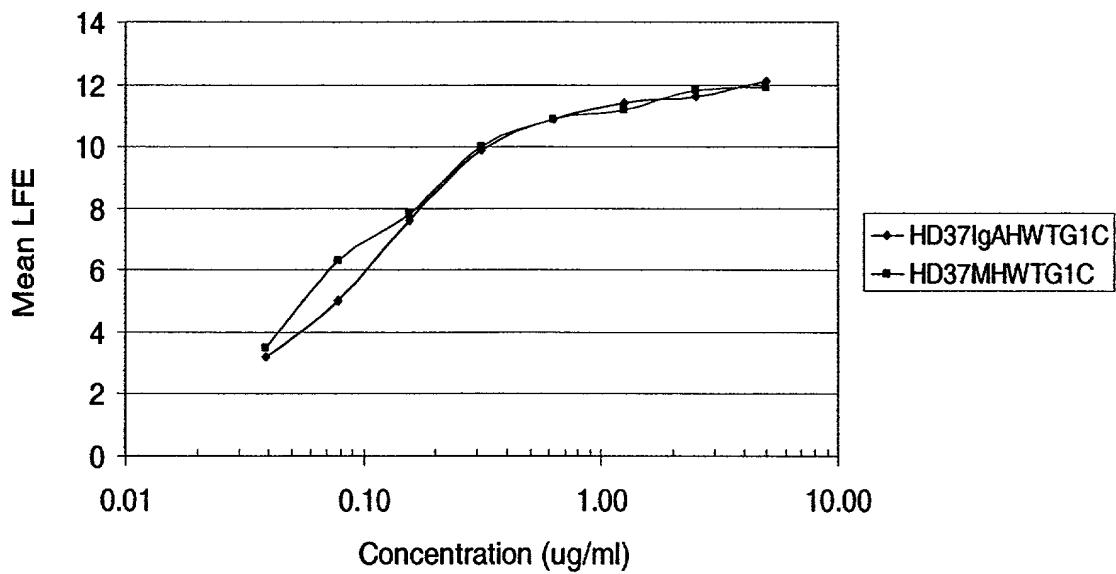


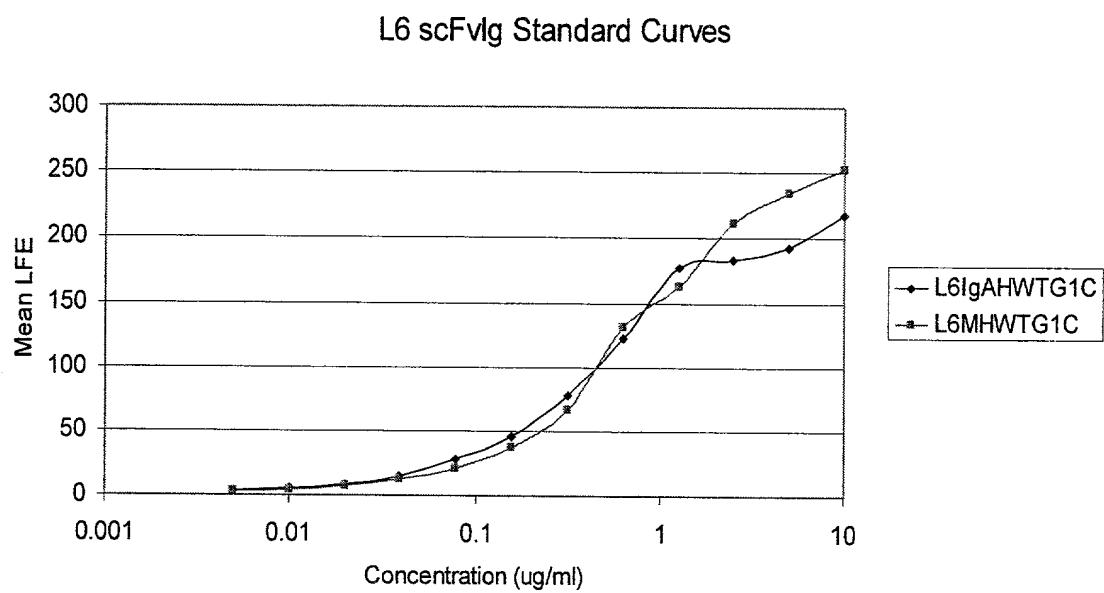
FIGURE 17

**Standard Curve of HD37 scFvIg Derivative  
Binding to B Cells**



Clone/Isolate	Mean LFE at 1:100	Estimated Concentration
Bulk IgAHWTG1C	11.2	> 60 ug/ml
1B2	10.4	>50 ug/ml
6C5	10.5	>50 ug/ml
4B1	8.6	>40 ug/ml
Bulk MHWTG1C	10.9	> 50 ug/ml
2G8	10.6	> 50 ug/ml
3F3	8.3	>40 ug/ml
3D9	11.1	> 60 ug/ml

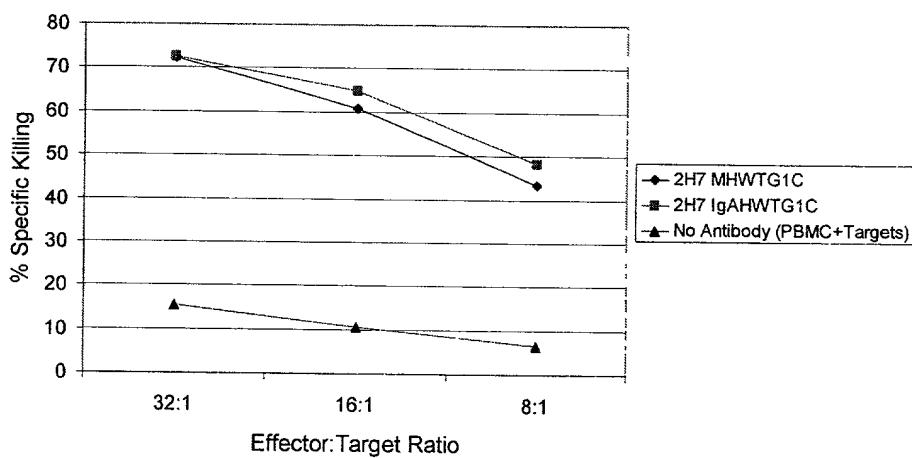
**FIGURE 18**



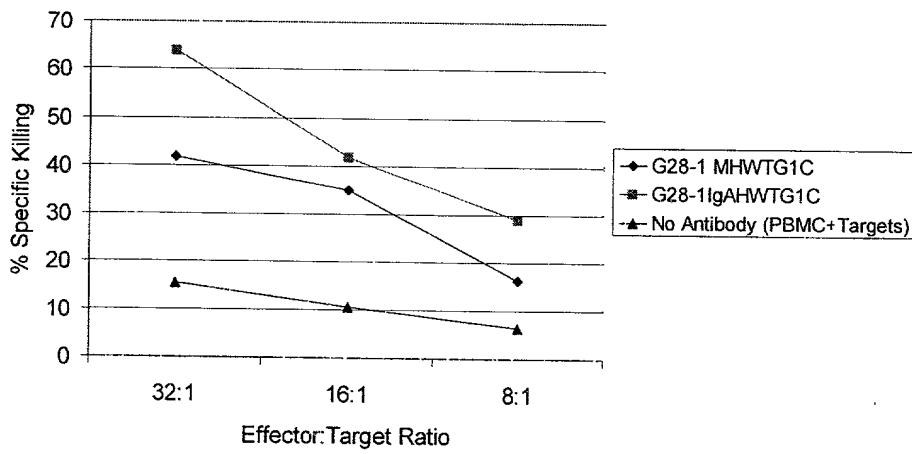
<u>Construct</u>	<u>Mean LFE 1:20</u>	<u>Estimated Concentration</u>
L6IgAHWTG1C unamplified CHO sup	51.1	6.25 ug/ml
L6IgGMHWTG1C unamplified CHO sup	23.0	3.2 ug/ml

**FIGURE 19**

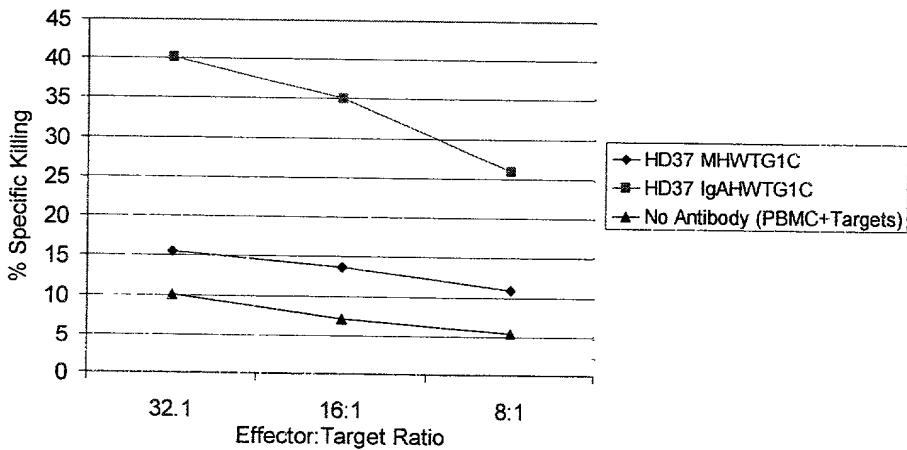
**A. 2H7 (anti-CD20) scFv Derivatives**



**B. G28-1 (anti-CD37) scFv Derivatives**



**C. HD37 (anti-CD19) scFv Derivatives**



**TITLE: BINDING DOMAIN-IMMUNOGLOBULIN FUSION PROTEINS**  
Inventors: Jeffrey A. Ledbetter et al. Docket No. 390069.401  
EXPRESS MAIL NO. EL 755733415US

FIGURE 20

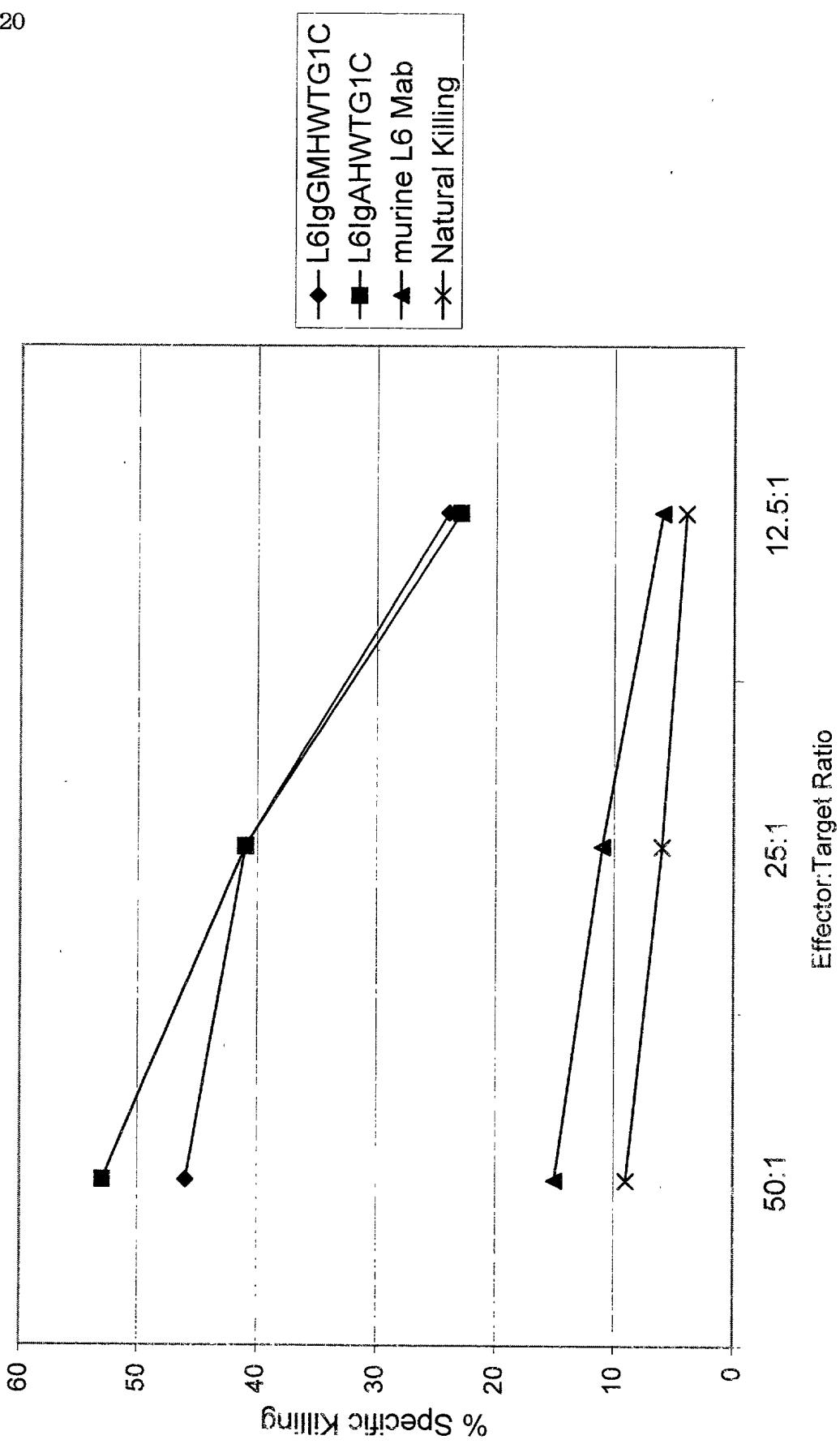


Figure 21

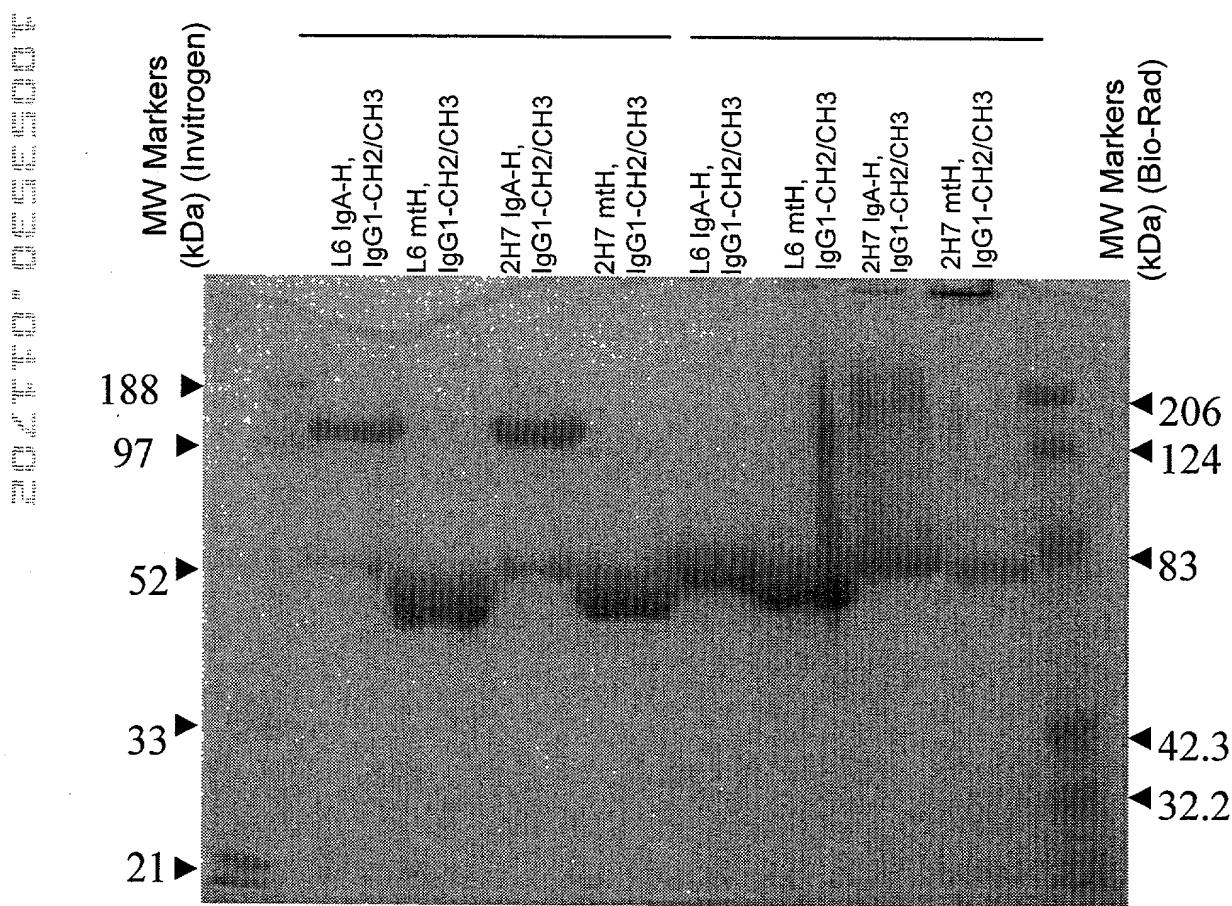


Figure 22

